REMARKS / ARGUMENTS

By the present amendment, claims 12, 34, 38 and 45 have been amended as described below, claim 33 has been cancelled and non-elected claims 43-44 and 50-52 have been withdrawn. The amendments to the claims have been made without prejudice and without acquiescing to any of the Examiner's objections. Applicants reserve the right to pursue any of the deleted subject matter in a further divisional, continuation or continuation-in-part application. No new matter has been entered by the present amendment and its entry is respectfully requested.

The office action dated July 22, 2009 has been carefully considered. It is believed that the amended claims and the following comments represent a complete response to the Examiner's rejections and place the present application in condition for allowance. Reconsideration is respectfully requested.

Elections/Restrictions

Applicant respectfully submits that elected Group I claims 1-26, 29-32, 34-42 and 45-49 are novel and inventive over the prior art cited by the Examiner for the reasons discussed below and thus, claims to non-elected species should not be withdrawn.

Specification

The Examiner objected to the specification as failing to provide proper antecedent basis for the claimed subject matter.

In particular, the Examiner objected to the specification as failing to provide antecedent basis for the claimed "removal in step (e) is performed by addition of the same antibody" as recited in claim 12. Applicant has amended claim 12 to replace "removal in step (e)" with "separation in step (f)" to correct for an obvious error.

The Examiner also objected to the specification as failing to provide antecedent basis for cross-linking the second ligand to the affinity matrix after separating the second ligand from the first ligand and before mixing the second ligand with a cellular lysate, as recited in claim 33. Claim 33 has been deleted rendering the Examiner's objection moot.

The Examiner objected to the specification as failing to provide antecedent basis for the limitations in claim 37. Applicant has amended the specification at paragraph [250] to include antecedent basis for this claimed subject matter.

In view of the above, Applicants respectfully request that the objections to the specification for lack of proper antecedent basis be withdrawn.

35 USC 112

The Examiner rejected claims 12, 34-35, 37-38 and 45-46 under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter.

In particular, the Examiner has rejected claim 12 as it recites the phrase "the removal in step (e) is performed by addition of the same antibody". According to the Examiner, it is unclear how adding the same antibody as the immobilized antibody would constitute the removal process. As stated above, Applicant has amended claim 12 to replace "removal in step (e)" with "separation in step (f)" to correct for an obvious error.

With respect to claim 34, the Examiner alleges that the phrase "and wherein the change of the concentration of the chemical or biomolecule is below 30 mM" is unclear because this phrase is located after the second "and/or" term. In response, claim 34 has been amended to insert a semi-colon prior to this phrase to clarify that the phrase qualifies both the prior phrases. Thus, Applicants submit that claim 34 and dependent claims 35 and 37-38 are definite.

With respect to claim 38, the Examiner alleges that the limitation "the mutated protein" in lines 4 and 5 and the limitation "the mutation" in line 5 lack sufficient antecedent basis. In response, Applicants have amended claim 38, to depend on claim 35 as suggested by the Examiner.

With respect to claim 45, the Examiner alleges that the phrase "protein-protein association" is unclear since claim 1 does not recite such an interaction. In response, claim 45 has been amended to replace "protein-protein association" with "ligand-ligand association" to be

consistent with claim 1. Thus, Applicants submit that claim 45 and dependent claim 46 are definite.

In view of the above, Applicants respectfully request that the rejections under 35 USC 112 be withdrawn.

35 USC 103(a)

The Examiner rejected claims 1, 32, 37 and 39-40 under 35 USC 103(a) as being unpatentable over US Patent Publication No. 2004/0142488 to Gierde et al. ("Gierde") in view of US Patent Publication No. 2004/0110675 to Sheehan. Applicants respectfully disagree for the reasons that follow.

Both Gierde and Sheehan teach to first purify the second ligand to homogeneity and then couple it to a solid support before applying biological sample containing the first ligand. Therefore, Gierde and Sheehan teach the formation *de novo* (*in vitro*) of an artificial complex between the first ligand and second ligand and sequential disruption of this artificially formed complex. The present inventors are the first to demonstrate that *in vivo* formed complexes are associated by predominantly electrostatic bonds and that the disruption of the electrostatic bonds is enough for the disintegration of the complex.

Further, Gierde uses classical affinity chromatography and teaches separation of the second ligand, i.e. the protein that binds to affinity matrix, from the affinity matrix.

In contrast, the presently claimed method requires that the first ligand be separated from the immobilized second ligand without causing the separation of the second ligand from the affinity matrix.

Thus, Gierde does not disclose or suggest step 1(d) separating the first ligand from the immobilized second ligand, which remains bound to the affinity matrix during the separation, by decreasing the electrostatic forces between the first ligand and the second ligand. The present inventors were the first to demonstrate that elimination of the dynamic range problem by separating the substoichiometrically interacting proteins (i.e. first ligand) from the immobilized fusion protein (i.e. second ligand that remains immobilized) is possible and desirable (see

patent application [0004-0015]). The cause for the failure to detect substoichiometrically interacting proteins was not known at the priority date of the application. For example, the weak interactors (i.e. first ligands) could have just been absent after immobilizing the protein of interest (i.e. second ligand) on affinity matrix or, they could have been present at amounts that did not allow their proper analysis and identification. The Applicants were the first to identify the cause for this failure, i.e. high dynamic range, and have designed a method which solves the problem. In particular, the following was unknown prior to the present application:

- transient interactors (i.e. first ligands) are present after the immobilization of the protein of interest (i.e. second ligand) on the affinity matrix but they are not detected because of the low stoichiometry (high dynamic range);
- transient interactors have to be separated from the high abundance affinity tagged protein and analyzed separately; and
- the separation of in vivo formed transient complexes can be achieved by increasing the ionic strength.

Only once the above was determined, could the presently claimed methods be developed.

The Examiner further alleges that Sheehan describes heparin-protein interactions as being dominated by electrostatic forces and that these forces can be disrupted by a NaCl gradient elution in an affinity column. Thus, the Examiner is of the opinion that one of ordinary skill in the art would have found it obvious to modify Gierde's method to investigate heparin-protein interactions using the affinity chromatography assay.

Applicant respectfully submits that if heparin were the second ligand in the Gierde method, it too would be eluted in response to a NaCl gradient, and thus the first ligand would not be separated from the second ligand, which remains immobilized as required by step 1(d). In particular, heparin is a very negatively charged biomolecule that will bind to the affinity matrix of Gierde by electrostatic forces and subsequently would be separated from the matrix during the NaCl elution (in parallel with the separation of protein of interest from heparin) and thus the presently claimed method would not be accomplished. (This is the reason why heparin is covalently bound to sepharose beads in commercial heparin-sepharose of Sheehan – see the bottom of page 1 of the attached reference from GE Healthcare). Thus, if one were to utilize the method of Gierde for the heparin-protein complexes of Sheehan, the NaCl elution would separate the first ligand from the second ligand but it would also lead to separation of heparin, i.e. the second

ligand, from the support material, which is in contrast to present step 1(d) where the first ligand is separated from the immobilized second ligand which remains bound to the affinity matrix.

Further, Sheehan states at [0189]: "The relative affinity of the recombinant proteins for heparin was assessed by the position of elution from a heparin-sepharose column in response to a NaCl gradient..." Thus, the heparin is not a second ligand in Sheehan but is itself part of the affinity matrix as it is covalently bound to the sepharose column.

In view of the above, Applicants respectfully submit that claims 1, 32, 37 and 39-40 are inventive over Gierde et al. and in view of Sheehan.

The Examiner rejected claims 1-11, 14-16, 18-19, 22-23, 34-35 and 38 under 35 USC 103(a) as being obvious having regard to Rigaut et al. (Nature Biotechnology (1999) 17:1030-1032) in view of Sheehan. Applicants respectfully disagree for the reasons that follow.

Rigaut teaches two purifications via an affinity tag performed one after another (Tandem Affinity Purification). Since purification via an affinity tag is a subdivision of affinity chromatography the arguments noted above with respect to Gierde et al. similarly apply to Rigaut. Rigaut analyzes isolated protein complexes by SDS-PAGE electrophoresis and thus teaches separation of the first ligand from the second by boiling in 1% SDS in presence of DTT which disrupts any known protein-protein interaction including the bond between the second ligand and the affinity matrix. This teaches away from the presently claimed method.

Rigaut does not teach or suggest step 1(d). As stated above, the present inventors were the first to demonstrate that elimination of the dynamic range problem by separating the substoichiometrically interacting proteins (i.e. first ligand) from the immobilized fusion protein (i.e. second ligand that remains immobilized) is possible and desirable (see the present application [0004-0015]).

The Examiner alleges that Sheehan describes heparin-protein interactions as being dominated by electrostatic forces and that these forces can be disrupted by a NaCl gradient elution in an affinity column. Thus, the Examiner is of the opinion that one of ordinary skill in the art would have found it obvious to modify Rigaut's method to investigate heparin-protein interactions using the tandem affinity purification assay.

(a) However, the method described by the Examiner on pages 9/10 would not work because heparin is a polysaccharide (more specifically glycosaminoglycan) but Rigaut's method is applicable to proteins only. I.e. the two tandem affinity purifications both include binding and separating protein from the affinity matrix by corresponding affinity tag. It is impossible to create recombinant heparin (i.e. heparin fused to an affinity tag) because there is no heparin gene that can be fused to DNA encoding affinity tag(s).

(b) Even if a skilled artisan manages somehow to create heparin coupled to an affinity tag (which would involve several additional inventions) still he would not be able to perform the presently claimed method. As discussed above, heparin is a very negatively charged biomolecule that will bind to the affinity matrix of Rigaut by electrostatic forces and subsequently would be separated from the matrix during the NaCl elution (in parallel with the separation of protein of interest from heparin) and thus the presently claimed method would not be accomplished. Thus, if it were possible to utilize the method of Rigaut for the heparin-protein complexes of Sheehan, the NaCl elution would separate the first ligand from the second ligand but it would also lead to separation of heparin, i.e. the second ligand, from the support material, which is in contrast to step 1(d) where the first ligand is separated from the immobilized second ligand which remains bound to the affinity matrix. This is the main reason why nobody (before the present disclosure or even up to this day) is trying to create heparin with attached affinity tag, i.e. the strong electrostatic interactions between the heparin and the affinity matrix make impossible the formation of specific bond(s) between the known affinity tags and the corresponding affinity matrices.

The presently claimed method solves an outstanding and long-recognized problem in the field of detecting protein-protein interactions, i.e. high dynamic range problem. There is a huge difference between the results obtained by using the presently claimed method and the prior art. There has always been a long-felt need to reliably isolate and identify proteins that interact with a protein(s) of interest. As an example, Gavin et al. (Nature, vol. 415, January 10, 2002, pp. 141-147 + Supplementary Table (enclosed)) used the method of "Rigaut" on a large scale (1,739 protein purifications) and, in particular, performed 3 protein purifications by using an affinity tag fused to 3 different subunits (Rpb3, Rpb7 and Rpb9) of RNA polymerase II, which is a permanent complex of twelve subunits (Rpb1-Rpb12). They report only 7 proteins that interact

with the permanent complex (See Supplementary table S1). By contrast, by using the presently claimed method, the present inventors were able to identify more than 70 additional interacting proteins (see present application at [0357] and Examples).

Moreover, Gavin et al. Nature (2002), while aware of Hata et al., (The Journal of Biological Chemistry, Vol. 268, No. 12, pp. 8447-8457 (1993), enclosed) which describes that heparin-protein interactions can be disrupted by a NaCl elution, performed 1,739 protein purifications but failed to realize that separation of the first ligand from the immobilized second ligand would greatly improve the final result. This demonstrates that the presently claimed method is non-obvious.

The Examiner rejected claims 13, 17 and 20-21 under 35 USC 103(a) as being obvious having regard to Rigaut in view of Sheehan and further in view of Gierde. As stated above, Rigaut in view of Sheehan do not teach or suggest affinity chromatography of *in vivo* formed complexes. Rigaut in view of Sheehan further do not teach or suggest step 1(d) separating the first ligand from the immobilized second ligand, which remains bound to the affinity matrix during the separation. Gierde et al. first purify the second ligand to homogeneity and then couple it to a solid support before applying biological sample containing the first ligand and also Gierde uses classical affinity chromatography and teaches separation of the second ligand, i.e. the protein that binds to affinity matrix, from the affinity matrix. Thus, Gierde et al. does not correct for the deficiencies of Rigaut in view of Sheehan.

The Examiner rejected claim 24 under 35 USC 103(a) as being obvious having regard to Rigaut in view of Sheehan, as applied to claims 1 and 22-23 above, and further in view of US Patent No. 5,007,934 to Stone and US Patent No. 5, 849,885 to Nuyens et al. As discussed above, Rigaut in view of Sheehan does not teach or suggest affinity chromatography of *in vivo* formed complexes. Rigaut in view of Sheehan further do not teach or suggest step 1(d) separating the first ligand from the immobilized second ligand, which remains bound to the affinity matrix during the separation. and separating the first ligand from the second ligand, which remains bound to the affinity matrix. The Examiner cites Stone as disclosing using NaCl or KCl as equivalent salts for removing glycoprotein or proteoglycan associated with collagen through electrostatic interaction and cites Nuyens as disclosing NaCl or KCl as equivalent salts for reducing electrostatic interactions between lactoferrin and other proteins. Thus, neither Stone nor Nuyens correct for the deficiencies discussed above of Rigaut in view of Sheehan.

The Examiner rejected claims 25 and 26 under 35 USC 103(a) as being obvious having regard to Rigaut in view of Sheehan as applied to claims 1 and 22-23 above. As discussed above, Rigaut in view of Sheehan does not teach or suggest affinity chromatography of *in vivo* formed complexes. Rigaut in view of Sheehan further do not teach or suggest step 1(d) separating the first ligand from the immobilized second ligand, which remains bound to the affinity matrix during the separation. and separating the first ligand from the second ligand, which remains bound to the affinity matrix. Thus, the fact that a person of ordinary skill in the art may be able to select a change in concentration does not correct for the deficiencies discussed above of Rigaut in view of Sheehan.

The Examiner rejected claims 45 and 46 under 35 USC 103(a) as being obvious having regard to Rigaut in view of Sheehan as applied to claim 1 above, and further in view of Patent 6,610,508 to Hentze et al. As discussed above, Rigaut in view of Sheehan does not teach or suggest affinity chromatography of *in vivo* formed complexes. Rigaut in view of Sheehan further do not teach or suggest step 1(d) separating the first ligand from the immobilized second ligand, which remains bound to the affinity matrix during the separation. The Examiner alleges that a person skilled in the art would have found it obvious to modify Rigaut and Sheehan's method to include the step of identifying protein-protein interactions for detecting Alzheimer's disease and that there would be a reasonable expectation of success because protein-protein interaction is a type of ligand-receptor interaction, which is known to be a reversible electrostatic attraction as evidenced by US Patent No. 5,753,225 to Clary et al. The fact that Hentze discloses a step of identifying protein-protein interactions in order to detect disease states including Alzheimer's Disease does not correct for the deficiencies discussed above of Rigaut in view of Sheehan. Further, the Examiner alleges that "Clary describes receptor-ligand complexes as reversible electrostatic attractions (225 Patent, col 10 II.66-67; col.11 II.1-11)". In fact, Clary states that *"Typically, the binding interactions between ligand or peptide and receptor or antigen include reversible noncovalent associations such as electrostatic attraction, Van der Waals forces and hydrogen bonds"*. The same statement is true for the permanent protein complexes, as well. Clary does not formulate that reversible protein complexes are held together by predominantly electrostatic forces and that disrupting the electrostatic forces leads to dissociation of the complex. Skilled artisan would consider that hydrophobic forces, Van der Waals forces and hydrogen bonds are not affected by the salt concentrations that significantly weaken and/or disrupt electrostatic attractions and would expect that disrupting the electrostatic forces is not

enough for dissociation of the protein complex. Thus the skilled artisan would have no motivation to try separating the first ligand from the immobilized second ligand.

In view of the above, Applicants respectfully submit that the present claims are inventive over Rigaut in view of Sheehan alone or having regard further to Hentze, Stone, Nuyens or Gierde.

In view of the foregoing, Applicants respectfully request that the rejections under 35 USC 103(a) be withdrawn.

The Commissioner is hereby authorized to charge any fee (including any claim fee) which may be required to our Deposit Account No. 02-2095.

In view of the foregoing comments and amendments, we respectfully submit that the application is in order for allowance and early indication of that effect is respectfully requested. Should the Examiner deem it beneficial to discuss the application in greater detail, he is kindly requested to contact the undersigned by telephone at (416) 957-1678 at his convenience.

Respectfully submitted,

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